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Poly(A) on Mengovirus RNA

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The content and size of the poly(A) on Mengovirus RNA grown in both mouse L cells and HeLa cells have been examined. Virion RNA from either cell line could bind to poly(U) filters and contained RNase-resistant stretches of poly(A) which could be analyzed by electrophoresis in polyacrylamide gels. The size of the poly(A) on the Mengovirus RNA was independent of the host cell and averaged from 50 to 70 nucleotides.

Sequences of poly(A) are found covalently linked to both cellular and viral messenger RNAs (see references 3 and 13 for review). In the picornavirus group, the genomes of poliovirus (1, 12, 15), encephalomyocarditis virus (4), Columbia S-K virus (6), Mengovirus (8), and rhinovirus (9) all contain a sequence of poly(A). The size of the poly(A) on these viruses, with the exception of Mengovirus, ranges from 50 to 100 nucleotides in length. R. L. Miller and P. G. W. Plagemann found that the size of the poly(A) on Mengovirus RNA grown in Novikoff rat hepatoma cells (8) or L cells (Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, V 28, p. 205) was only an average of 15 to 17 nucleotides in length. Previously we had found that the 3'-terminal poliovirus poly(A) serves a critical biological function because severe reduction of the size of the poly(A) by RNase H markedly decreased the specific infectivity of the poliovirus RNA molecules in HeLa cells (11). In order to determine whether the minimal length of poly(A) necessary for the replication of a picornavirus was dependent on the host cell, we studied the size of the poly(A) on Mengovirus RNA grown in both L cells and HeLa cells.

The growth of suspended HeLa cells in Joklik modified minimal essential medium plus 7% horse serum, the production of type 1 poliovirus, and the purification of virion RNA labeled with [2,8-^{3}H]adenosine have been described (2, 12). To prepare Mengovirus RNA, cultures of 4×10^8 HeLa and L cells were washed once with Earle saline and infected by suspending at 5×10^7 cells/ml in medium adjusted to 40 mM $MgCl_2$ and containing 2 PFU of Mengovirus per cell (kindly supplied by Peter Plagemann of the University of Minnesota). The Mengovirus stock had been prepared by growth in Novikoff rat hepatoma cells as described previously (8). After adsorption at room temperature for 60 min, the cells were diluted to 4×10^6/ml with warm medium containing 5% fetal calf serum. The suspensions were supplemented with 1 μg of actinomycin D per ml (kindly supplied by Merck, Sharp and Dohme) 30 min after infection and with 100 μCi of [2.8-^{3}H]adenosine or [5,6-^{3}H]uridine (New England Nuclear) per ml at 1 h after infection. At 7.5 h after infection the virus was purified according to the procedure of Miller and Plagemann (8). RNA was extracted and purified from the virus as described for poliovirus RNA (12). The procedures for the poly(U) binding of viral RNA, for T_1 plus pancreatic RNase digestion of RNA, and for the isolation and polyacrylamide gel electrophoresis of poly(A) have been described in detail previously (11).

To investigate whether Mengovirus RNA grown in either L cells or HeLa cells contained poly(A), the capacity of these RNA species to bind to poly(U) filters was studied (Table 1). ^{3}H-labeled poly(A) itself bound with 100% efficiency. The Mengovirus RNA grown in HeLa cells bound with an efficiency of 80% while that grown in L cells bound with an efficiency of 67%. These values were similar to the binding efficiency of poliovirus RNA (63%) and represent minimum values of binding because it has been shown for poliovirus RNA that virion RNA which passes through a poly(U) filter contains the same size and amount of poly(A) as the RNA retained by the filter (12).

To determine whether the poly(A) was covalently bound to the 35*S* virion RNA of poliovirus and of Mengovirus, [^{3}H]adenosine-labeled poliovirus RNA and Mengovirus RNA grown in both HeLa cells and L cells were denatured with dimethyl sulfoxide and sedimented through 5 to

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20% sucrose gradients in 80% dimethyl sulfoxide, 10 mM Tris-hydrochloride, pH 7.0, 10 mM LiCl, and 1 mM EDTA. Samples from the 35S peak fraction were assayed for their ability to bind to poly(U) filters (Table 1, values in parenthesis). The dimethyl sulfoxide-denatured viral RNAs of poliovirus and Mengovirus bound to poly(U) with efficiencies essentially equal to those of the native RNA, indicating that both contained covalently bound poly(A).

A second method for assaying poly(A) content of RNAs is determination of the fraction of RNA resistant to pancreatic plus T_1 RNases (Table 1). ^{3}H-labeled poly(A) was completely nuclease resistant and [^{3}H]adenosine poliovirus RNA was found to have an acid-precipitable, RNase-resistant fraction representing 3.8% of the total [^{3}H]AMP label. Since poliovirus RNA contains 7,500 nucleotides of which 29% are AMP, 3.8% of the [^{3}H]adenosine label is RNase resistant and this corresponds to 87 nucleotides of AMP in poly(A) per molecule. [^{3}H]adenosine-labeled Mengovirus RNA contained slightly less RNase-resistant material than poliovirus RNA (3.5% when grown in HeLa cells and 3.3% when grown in L cells).

To investigate the size of the poly(A) sequences on Mengovirus and poliovirus RNA, the RNA which resisted digestion by pancreatic plus T_1 RNases was bound to poly(U) filters, eluted, and subjected to electrophoresis in 10% polyacrylamide gels (Fig. 1). The poly(A) from

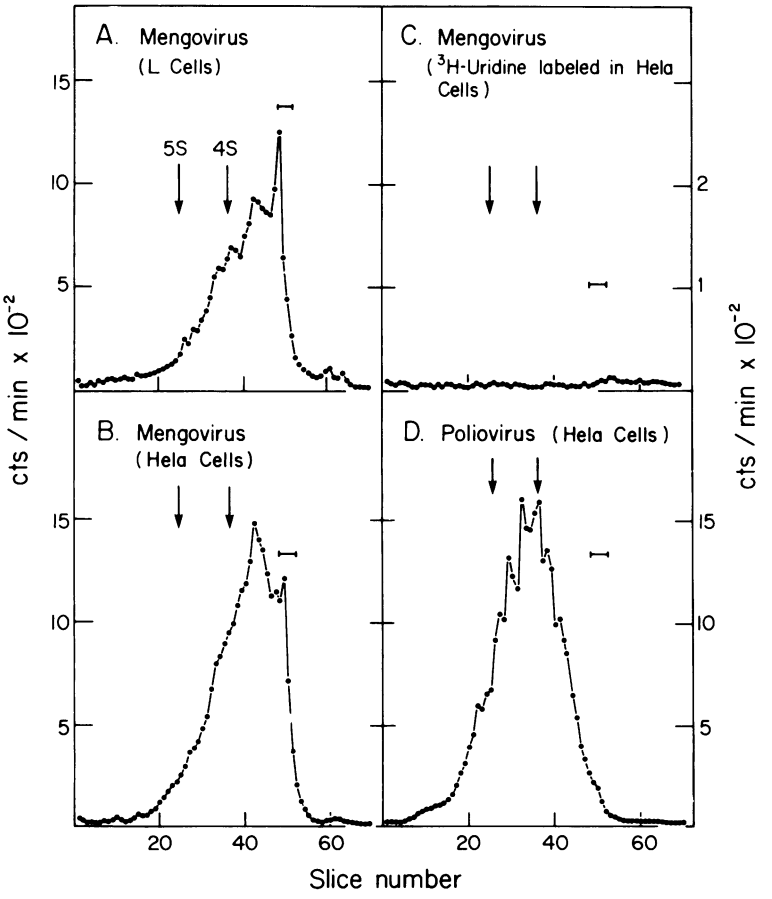

FIG. 1. *Polyacrylamide gel electrophoresis of poly(A) sequences in continuously labeled Mengovirus and poliovirus RNA. The RNA samples were treated with T_1 plus pancreatic RNases, bound to poly(U) filters, and eluted. The ethanol-precipitated poly(A) was dissolved in 100 μl of 50% formamide, 25% glycerol, 0.04 M Tris, pH 7.2, 0.02 M sodium acetate, 0.001 M EDTA, and 0.2% sodium dodecyl sulfate, and electrophoresed in 10% polyacrylamide gels at 7.5 mA/gel for 5 h. Slices (2 mm) of the gel were directly counted in a toluene-based scintillation fluid containing 3.5% Nuclear-Chicago solubilizer. HeLa 4S and 5S RNAs served as markers. The horizontal bar indicates the position of the bromophenol blue dye marker. Panels: (A) [^{3}H]adenosine Mengovirus RNA grown in L cells; (B) [^{3}H]adenosine Mengovirus RNA grown in HeLa cells; (C) [^{3}H]uridine Mengovirus RNA grown in HeLa cells; and (D) [^{3}H]adenosine poliovirus RNA grown in HeLa cells.*

TABLE 1. *Poly(U) binding and RNase resistance of RNA species*[a]

RNA species	Host cell	% Bound to poly(U) filters	% Resistant to T_1 plus pancreatic RNases
[^{3}H]adenosine Mengovirus RNA	HeLa	80 (84)	3.5
	L	67 (70)	3.3
[^{3}H]adenosine poliovirus RNA	HeLa	63 (77)	3.8
Poly(A)		100	100

[a] To determine the percentage of resistance to T_1 plus pancreatic RNases 0.1 ml of each sample in 0.1% sodium dodecyl sulfate buffer (0.01 M Tris-hydrochloride, pH 7.5, 0.1 M NaCl, 0.001 M EDTA, 0.1% sodium dodecyl sulfate) was incubated in 4 ml of a solution containing 0.3 M NaCl, 0.03 M sodium acetate, 10 μg of pancreatic RNase per ml, and 10 U of T_1 RNase per ml for 60 min at 37 C. The samples were then precipitated with 12.5% trichloroacetic acid. Values of the percentage of binding to poly(U) filters and of the percentage of resistance to T_1 plus pancreatic RNases represent the average of at least two independent determinations. The numbers in parenthesis indicate the percentage of binding to poly(U) filters of RNA samples from the 35*S* peak fractions of a 5 to 20% sucrose gradient in 80% dimethylsulfoxide 10 mM Tris-hydrochloride, pH 7.0, 10 mM LiCl. and 1 mM EDTA.

poliovirus RNA was heterogeneous (Fig. 1D). From the position of the 4*S* and 5*S* marker it was estimated that the poly(A) has approximately 50 to 125 nucleotides. This estimate agrees both with the percentage of [^{3}H]adenosine resistant to RNase (indicating 87 nucleotides) and with an estimate of 71 nucleotides which was obtained by determining the ratios of AMP to adenosine in the poly(A) (12). As indicated by the fraction of [^{3}H]adenosine-labeled Mengovirus RNA resistant to T_1 plus pancreatic RNases (Table 1), the size of the poly(A) on Mengovirus RNA whether grown in L cells (Fig. 1A) or HeLa cells (Fig. 1B) was slightly smaller than poliovirus poly(A), with an electrophoretic mobility corresponding to an average of 50 to 70 nucleotides in length. As expected, from the [^{3}H]uridine-labeled Mengovirus RNA no labeled oligonucleotides were detected by gel electrophoresis after RNase digestion and poly(U) selection (Fig. 1C).

The results presented here demonstrate that the poly(A) on Mengovirus, whether the virus is grown in L cells or HeLa cells, is only slightly smaller than that on poliovirus RNA with an average length of 50 to 70 nucleotides. The finding that the genomes of poliovirus (1, 12, 15), encephalomyocarditis virus (4), Columbia S-K virus (6), and rhinovirus (9) all contain a sequence of poly(A) ranging from 50 to 100 nucleotides in length coupled with the results presented here add further evidence that the picornaviruses do not differ markedly in the size of their poly(A). Since we have previously demonstrated that reduction of the size of the poly(A) on poliovirus RNA below 50 nucleotides markedly decreased the specific infectivity of the RNA, it is likely that a minimum of 40 to 50 nucleotides of poly(A) is necessary for the replication of picornaviruses.

Our findings are in direct disagreement with those of Miller and Plagemann (8). They calculated that the poly(A) on Mengovirus RNA grown in Novikoff rat hepatoma cells or in mouse L cells was only 15 to 17 nucleotides in length. The reasons for this discrepancy are unknown, but it is possible that their method of phenol extraction of the RNA resulted in the loss of some of the poly(A) as has been reported by Perry et al. (10).

Whereas it is possible that the size of the poly(A) is somehow determined by physiological parameters of the cells which differed for the cells grown in their laboratory and ours, this possibility seems unlikely. In the case of poliovirus, the poly(A) seems to be transcribed from a poly(U) sequence on the minus strand (D. H. Spector and D. Baltimore, Virology, in press; 14, 16, 17). Whereas the size of the poly(A) on the intracellular poliovirus-specific RNAs varies during the course of the infection (12), only molecules with poly(A) ranging from 50 to 125 nucleotides in length appear to be encapsidated.

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